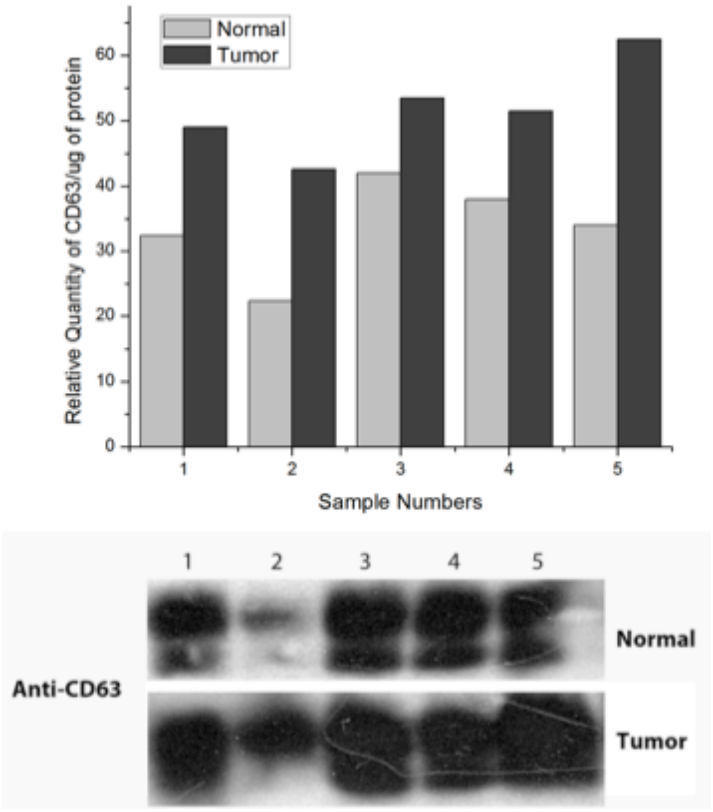
Supplementary:

Western Blots: Briefly, exosome proteins (1 mg/ml total protein) were extracted and separated on a polyacrylamide gel before transfer to a nitrocellulose membrane. The blotting membrane was blocked and incubated with CD63 (Santa Cruz Biotechnology, Santa Cruz, CA) antibody followed by incubation with horseradish peroxidase coupled secondary antibody. The proteins were detected using enhanced chemiluminescence.

AFM imaging: Tapping mode imaging detects the change in the vibration amplitude of the oscillating cantilever and uses it as a feedback signal to generate high-resolution 3D topographic (height) images. Phase images in which the phase change of the cantilever relative to the excitation signal is recorded while the feedback maintains constant vibration amplitude of the cantilever allows mapping variations in material properties such as exosomes density and visco-elasticity.

Exosomes size analysis: The size of isolated individual salivary exosomes was determined from AFM height images. Images were zero-order flatten, plane fit and using grain analysis function, the particle size of isolated exosomes were measured (SPIP Version 4.8.2). Using particle size analysis, the nanoscale diameters of exosomes were measured from AFM topographic images. 10ul of diluted samples (1:200 from 1mg/ml concentration of protein stock) were added to mica surface and 8x8um scans were obtained at different regions of the sample to determine the distribution and average vesicle dimensions at the single exosomes level.

Single Molecule Force Spectroscopy: The AFM tip was positioned over the top of the vesicle by first imaging the vesicles in tapping mode to localize the particles and using point and shoot module (Nanoscope 8.1) to obtain force curves over the exosomes. Force–displacement curves were recorded over >10 individual exosomes under PBS conditions, at 1 Hz. All measurements were obtained under buffer conditions over exosomes immobilized over mica surface at room temperature. Non-specific mouse antibody coated AFM tips were used as control. Single isolated exosomes immobilized to mica surface under buffer conditions were imaged at low forces using tapping mode, to localize the vesicles and also ascertain that the force curves were obtained over single exosomes rather than multivesicular bodies enclosing the exosomes. The antibody functionalized tips were repeatedly brought into contact with the surface of the exosomes to facilitate interaction and retracted away from the surface to measure the binding force interaction with specific biomolecular exosome surface receptors present on the external vesicle surface.



Supplementary Figure 1. Relative protein concentration and western blots for all saliva exosomes samples.